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The Pathology of Myxosporidiosis in the Electric Eel, *Electrophorus electricus* (Linnaeus), Caused by *Henneguya visceralis* and *H. electrica* spp. nov.

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(Plates I-VI; Text-figures 1-4)

INTRODUCTION

MYXOSPORIDIANS, which are predominantly fish parasites, have been reported as infecting practically every tissue and organ. Many species are host and tissue specific, or limited in occurrence to certain families of fishes in definite localities. There is very little information, however, concerning the pathology associated with these sporozoan infections. This contribution deals with a detailed description of two of three species of myxosporidians found in the electric eel, which were previously recorded but not named by Jakowska & Nigrelli (1950); these parasites are considered new and are here named *Henneguya visceralis* and *Henneguya electrica* spp. nov. Their distribution in the host and their effects on the tissues are described and discussed.

MATERIAL AND METHODS

Forty-one electric eels, *Electrophorus electricus* (Linnaeus), ranging in size from 8 to 40 inches, were examined shortly after death. The animals had been in captivity at the New York Aquarium for periods ranging from a few days to many months. The organs showing myxosporidian cysts were fixed in Bouin's fluid or in formalin, sectioned at 5 microns and stained with hematoxylin-eosin, Giemsa's and Masson's methods.

All measurements of mature spores (Table 1) were made on fresh material obtained by teasing out the cysts in saline or in distilled water, and then studied in coverslip or hanging drop preparations under 20 × ocular and 43 × objectives. Camera lucida drawings were made from these materials. In addition to sections and wet mounts, touch preparations from hemopoietic organs in which myxosporidians occurred were stained with Wright's method. Vegetative stages were described from sectioned material.

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OBSERVATIONS

Description of New Species of *Henneguya*

On the basis of the unfixed mature spores, two types of *Henneguya* were sufficiently distinct from other described Brazilian members of this genus (Table 1) to be considered as new species. No distinction, however, could be made on the basis of the size, shape, color and consistency of the cysts. There was no apparent relationship either between the degree of infection and maturity of the host or between the microscopical characteristics of the cysts and the developmental stages of the parasites. In every case examined, it was found, however, that the parasites in each cyst had attained the same stage of development, i.e., either sporogony was almost completed or the cysts contained vegetative stages only.

TABLE 1. COMPARISON OF *H. visceralis* AND *H. electrica* WITH OTHER SPECIES OF *Henneguya* FROM BRAZILIAN FRESHWATER FISHES

Data Concerning Each Species: Name of Species, Discoverer, Host Fish, Infected Tissues	Spore Measurements in Microns					
	Total Length	Body Length	Body Width	Extension Length	Polar Capsule Length	Polar Capsule Width
H. linearis (Gurley, 1893) in *Rhamdia sebae* and *Pseudoplatystoma fasciatum* Branchial cavity, gills	Insuff. data	3× or 4× the width	Very narrow	No data	No data	No data
H. lutzi (Cunha & Fonseca, 1918) in *Pseudopimelodus zungaro* Gall bladder	11	No data	7	22	6-7	No data
H. occulta (Nemeczek, 1926) in *Loricaria* sp. Gills	36-46 Max. 50	16-20	8-10	20	8	No data
H. leporini (Nemeczek, 1926) in *Leporinus mormyrops* Urinary ducts	28-33	13-15	5	15-18	5-8	No data
H. wenyoni (Pinto, 1928) in *Astyanax fasciatus* Gills and intestine	20 19-24	11-12	4.5-6	8-12	3.4-5	1.5
H. iheringi (Pinto, 1928) in *Serrasalmo spiropleura* Gills	22	Tapered	6	None	2.4	2
H. fonsecai (Guimaraes, 1931) in *Leporinus copelandi* Cutaneous tissues of fins	23-27	10-12	4.5-5	13-15	4-4.2	No data
H. cesarpintoi (Guimaraes, 1931) in *Astyanax fasciatus* Branchial cavity	13-14	5.5-6	4-4.5	7.5-8	2.5	0.8
H. bergamini (Guimaraes, 1931) in *Astyanax fasciatus* "General" cavity	17-19	7-8	2-2.5	10-11	No data	No data
H. travassosi (Guimaraes & Bergamin, 1933) in *Astyanax fasciatus and Leporinus copelandi* Muscles and cutaneous tissues	26.3-28	10.1-10.8	3.8-4.8	15.3-18	3.2-4.2	No data
H. santae (Guimaraes & Bergamin, 1934) in *Tetragnopterus santae* Gills and branchial arches	19.3-22.7	8.5-10.6	4.7-5.8	10.4-12.7 (8.7)	2.5-3.5	No data
H. visceralis (Jakowska & Nigrelli) in *Electrophorus electricus* Kidney, liver, heart, mesentery	22-24	11-12	5-6.5	11-12	6.5-8	2
H. electrica (Jakowska & Nigrelli) in *Electrophorus electricus* Large electric organs	35-39	11-13	6-8	24-27	5-7	2
Henneguya spp. (?) (Jakowska & Nigrelli) in *Electrophorus electricus* Oral mucosa and upper lip, skin	35-38	11-13	2.5	24-25	3.5	No data

Diagnosis of Species

The diagnosis of species of myxosporidians is based primarily on spore size and shape, dimensions of polar capsules and presence or absence of specialized structures. On these characters alone, *Henneguya visceralis* and *Henneguya electrica* differ from each other and from other recognized species.

The spore of *H. visceralis* measures 22-24 microns in total length and 5.0-6.5 microns in width. The length of the body is 11-12 microns and the posterior extensions equal this length. The polar capsules are equal in size, 6.5-8 microns long and 2.0 microns wide. A comparison of these measurements with those of other species listed in Table 1 shows that the only species to approximate them is *H. wenyoni;* it differs, however, from *H. visceralis* in the dimensions of the polar capsules, which are smaller, measuring 3.4-5 × 1.5 microns. *H. iheringi* has a body length similar to that of *H. visceralis,* but lacks the posterior extensions.

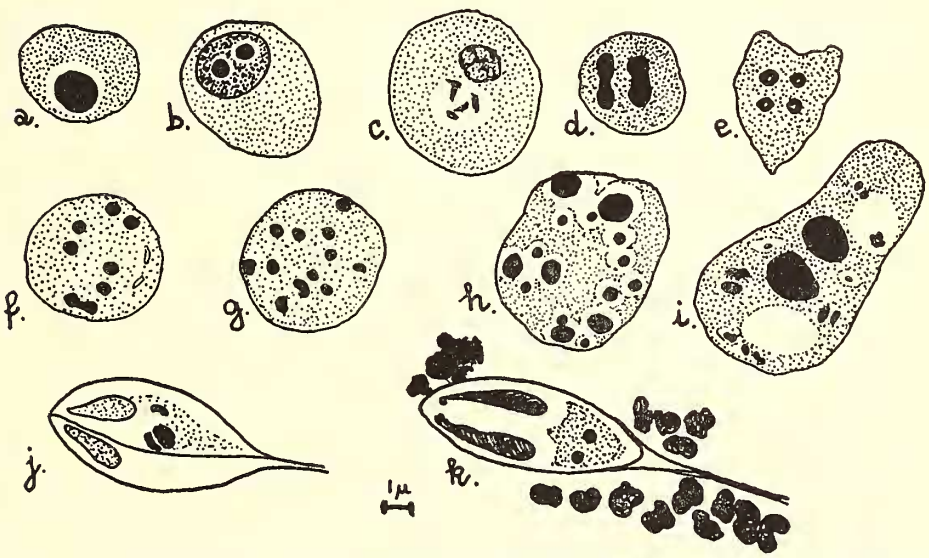
The spore of *H. electrica* measures 35-39 microns in total length and 6-8 microns in width. The body proper measures 11-13 microns in length, while the posterior extensions are considerably longer (24-27 microns). The polar capsules measure 5-7 × 2 microns. The total body length of *H. electrica* falls within the size range shown for *H. occulta* in Table 1; the latter species, however, possesses shorter posterior extensions, about equal to the length of the body.

The main distinguishing feature between the spores of *H. visceralis* and *H. electrica* consists, among others, in the different length of the posterior extensions which measure 24-27 microns in *H. electrica* and 11-12 microns in *H. visceralis.*

Henneguya visceralis sp. n.

Except in the kidney, no early vegetative stages were found in cysts occurring in the stomach, liver, spleen, gall bladder and heart. Various stages of development leading to sporogony were found in the cysts of the kidney (Plate III, fig. 5) or free in the intertubular and interglomerular areas, where they were usually associated with the extra-cellular yellowish, siderin-like deposits (Plate I, figs. 1 and 2, and Plate II, figs. 3 and 4).

Description of the Vegetative Stages (Text-fig. 1, a-i).—Uninucleate trophozoites were of

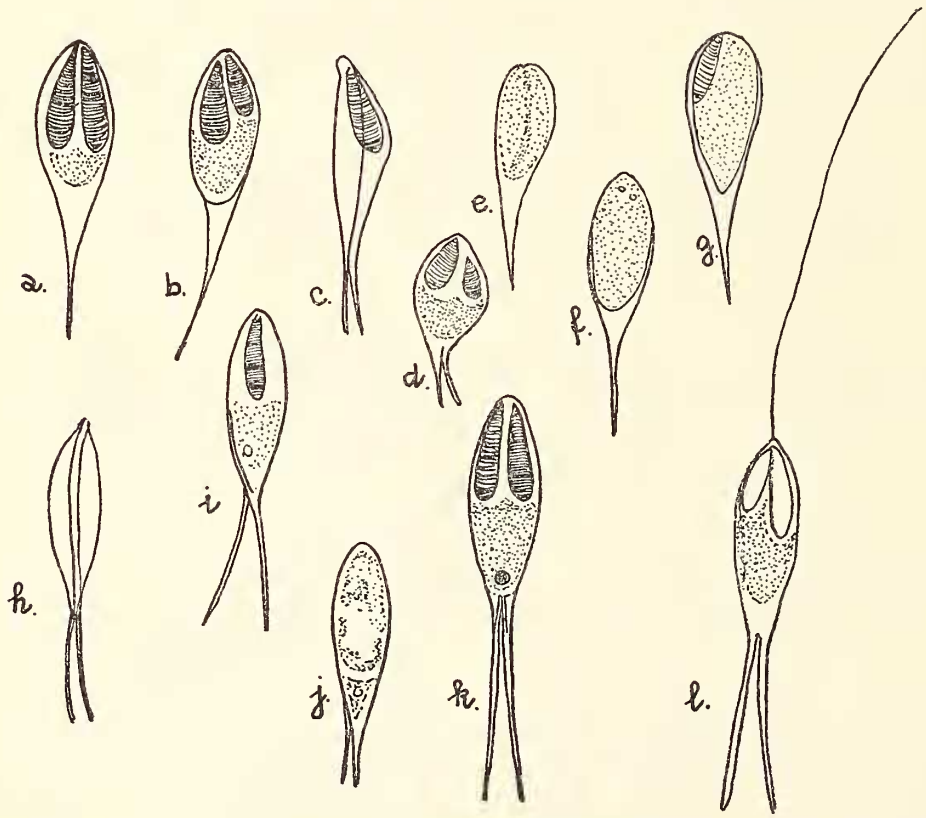


Text-fig. 1. Vegetative stages of *Henneguya visceralis* sp. n., together with an immature and mature spore, from a section of a superficial cyst located on the posterior kidney. Masson's stain. Appr. 4000 ×. *a*—uninucleate trophozoite with granular cytoplasm and a pycnotic nucleus surrounded by a clear perinuclear zone. *b*—uninucleate trophozoite with two nucleoli and a less granular cytoplasm. *c*—binucleate sporoblast suggesting a mitotic division in one of the two nuclei. *d*—binucleate sporoblast with cleaving nuclei suggesting amitosis. *e*—tetranucleate sporoblast; note the reduction in size of the nuclei. *f*—octonucleate sporoblast; the cytoplasm shows lesser granularity than in the earlier stages of development. *g*—sporoblast with twelve generative nuclei. *h*—initiation of spore formation in a pansporoblast; vacuole-like areas appearing around the nuclei of varying sizes. *i*—disporous pansporoblast showing a more symmetrical arrangement of vacuoles and nuclei. *j*—immature spore; posterior extension not developed. *k*—mature spore showing two sporoplasm nuclei; note the size of extracellular yellowish bodies (hemosiderin?).

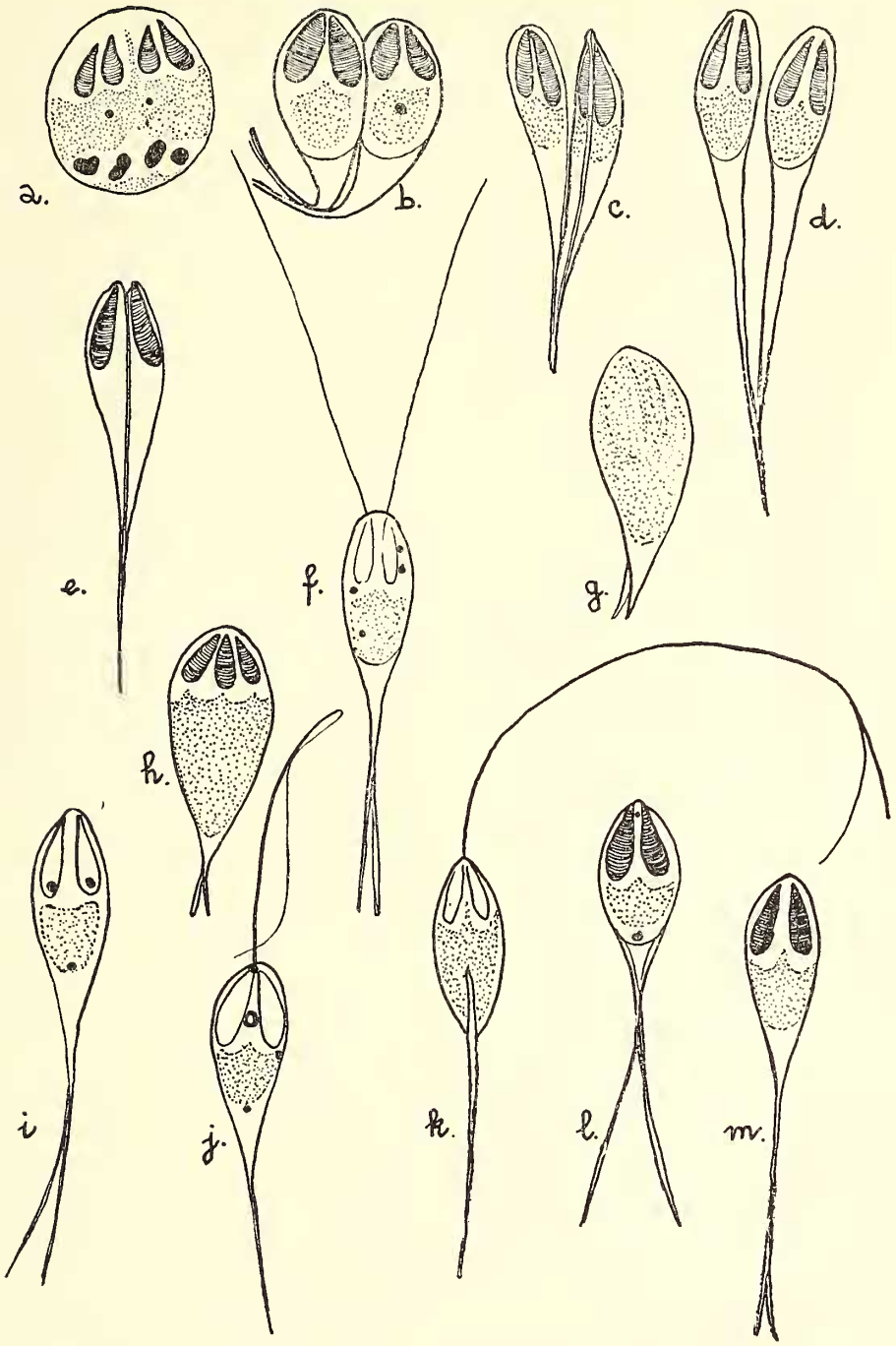
irregular shape, 4-9 microns in the wider dimension; the cytoplasm was granular, staining grayish with Masson's; nucleus pycnotic, surrounded by a clear granule-free zone (Text-fig. 1, a). Some uninucleate sporoblasts believed to be under transition to the next stage showed two nucleoli within a light staining nucleus (Text-fig. 1, b). Binucleate stages showed no conspicuous granule-free zone around the nuclei. There was some indication of mitotic division in binucleate sporoblasts (Text-fig. 1, c), but nuclear conditions suggesting amitosis were also observed (Text-fig. 1, d). Tetranucleate sporoblasts (Text-fig. 1, e and f) and sporoblasts with 12 generative nuclei (Text-fig. 1, g) had clearer cytoplasm. Pansporoblasts in which spore formation was initiated showed granule-free vacuole-like areas around the nuelei (Text-fig. 1, h). Disporous pansporoblasts (Text-fig. 1, i), 11 microns in the wider dimensions, exhibited a more symmetrical arrangement of vacuoles and nuclei. Immature spores (Text-fig. 1, j) had short posterior extensions.

Description of Cysts.—The surface cysts of *H. visceralis* were found in the kidneys of all the eels examined (100%) and the most common locus was the lateral margin of the posterior kidney. These were flat or raised, solid, white cysts. The cysts on the ventral and dorsal surfaces of the kidney were usually flat and milky in consisteney and sometimes vascularized. Less frequently, the solid white cysts were also found on the mesentary (sometimes associated with larval nematodes), on the surface of the stomach, liver, spleen, gall bladder and heart. Microscopically, however, cysts of this species also occurred in the deeper tissues of the above-mentioned organs and the pathology associated with these infections is described below. Yellowish soft cysts were occasionally seen, but their condition was attributed to *post-mortem* changes.

Description of Spores (Text-fig. 1, j, k; Text-



TEXT-FIG. 2. Unfixed spores of *Henneguya visceralis* sp. n. from a teased cyst on the kidney. Hanging drop preparation. Appr. 2000 ×, with the exception of figs. *k* and *l* which are magnified appr. 2500 ×. *a, b* and *c*—different aspects of mature spores; in *c* the space occupied by the sporoplasm was not determined. *d*—abnormally short, seemingly mature spore. *e, f* and *g*—immature spores. *h*—lateral view of an apparently empty spore case, showing smooth suture. *i*—oblique view of a mature spore. *j*—immature spore. *k*—mature spore showing a single sporoplasm nucleus (synkaryon). *l*—mature spore with discharged polar capsules; one filament extending anteriorly.



TEXT-FIG. 3. Unfixed young and mature spores of *Henneguya electrica* sp. n. from a teased cyst located on the dorsal margin of the large electric organ; hanging drop preparation. Appr. 2000 ×. *a*—nearly mature disporous pansporoblast. *b* and *c*—young spores emerging in paired condition; suture may be seen in *c*. *d*—a pair of mature spores joined at the tip of the posterior extension. *e*—lateral view of a pair of spores, showing their slightly compressed bodies. *f*—mature spore with discharged polar filaments. *g*—immature or abortive spore. *h*—early stage in the development of two apposed spores. *i*—mature spore showing empty polar capsules, capsule nuclei and a sporoplasm nucleus. *j* and *k*—mature spores with one polar filament extruded. *l* and *m*—typical mature spores.

fig. 2, a-l).—Mature spores with fully developed polar capsules and two sporoplasm nuclei (Text-fig. 1, k) were frequently found in the kidney tissue near the vegetative stages (Plate II, fig. 4, and Plate IV, fig. 7). More often they occurred in cysts on the kidney, stomach, liver, spleen, gall bladder, heart (Plate V, fig. 10), scrapings from the palate and from the lumen of the urinary duct. The spores, bodies measuring 11-12 × 5.0-6.5 × 4.5 microns (Table 1), were flattened on one side and slightly convex on the other (Text-fig. 2, c). Posterior extensions equaled the length of the body (Text-fig. 2, a and k). Polar capsules which measured 6.5-8 × 2 microns occasionally appeared asymmetrical in oblique view (Text-fig. 2, b). Extruded polar filaments measured up to 44 microns in length (Text-fig. 2, l). Some of the variation in shape, size and development of spores is shown in Text-fig. 1, j; Text-fig. 2, d-j.

Henneguya electrica sp. n.

Cysts of this form occurred on the median dorsal surface of the large electric organ (Plate VI, fig. 11). Vegetative stages were not observed. Some cysts contained terminal stages in sporogony (Text-fig. 3, a).

Description of Cysts.—The cysts of *H. electrica* were seen in only about 10% of the autopsies and macroscopically usually appeared similar to the solid white cysts of *H. visceralis*. Yellowish soft cysts were also found.

Description of Spores (Text-fig. 3, Plate VI, fig. 12)—Immature spores had broad bodies and short posterior extensions (Text-fig. 3, b, c and g). Mature spores (Plate I, figs. 1 and 2; Text-fig. 3, d-f, i-k, m), measuring 11-13 × 6-8 × 4.5 microns (Table 1), had nearly symmetrical valves; posterior extensions measured 24-27 microns; extruded filaments 44-50 microns (Text-fig. 3, f, g and k). The polar capsules occupied approximately one-half of the body length (5-7 microns). Fresh spores released from cysts retained their association in pairs, indicating that their development was synchronous (Plate VI, fig. 12; Text-fig. 3, b, c and d). Sporoplasm and capsular nuclei were evident in some spores (Text-fig. 3, i).

Henneguya spp. (?).

Gray thick cysts from the mouth of one electric eel and white solid cysts from the skin in the dorsal body region of several other specimens contained spores in various stages of development but no early vegetative stages were found. The spores from the mouth-cysts measured 11-13 × 2.5 microns; posterior extensions 24-25 microns. Polar capsules were approximately 3.5 microns long (Table 1; Text-fig. 4, g). The spores found in cysts on the skin were variable in size and shape, occasionally showing asymmetrical polar capsules (Text-fig. 4, b and d). These spores showed some similarities to *H. visceralis* and *H. electrica.*

Pathological Changes Associated with Myxosporidiosis in Electric Eels

Pathological manifestations caused by *Henneguya visceralis* were seen in the kidney, liver, spleen and heart, and the changes noted were probably the cause of death. The infection was partly characterized by the presence of numerous diffusely scattered circular or oval-shaped masses of siderin-like deposits. These were yellow in hematoxylin-eosin and Masson preparations and greenish when stained with Giemsa.

The posterior or excretory part of the kidney appeared to be the primary locus of the infection. Stages in sporogony were found in thin and thick-walled cysts situated at the surface and just below the surface (Plate III, fig. 5) and as a diffuse infiltration within the kidney tissue associated with siderin-like bodies (Plate I, figs. 1 and 2; Plate II, fig. 3). Uninucleate trophozoites, readily recognized in Masson preparations by their grayish appearance, were found scattered in the intertubular and interglomerular regions (Plate II, figs. 3 and 4). In these areas, the pathological reactions were manifested by the development of numerous sinusoids, fibrous tissue, tubercle-like concretions and extensive parenchymatous degeneration (Plate I, fig. 2; Plate II, fig. 4). There was evidence of macrophage activity in some parts of the kidney, but no eosinophilia or typical inflammatory reaction were seen. It could not be determined whether or not these pathological changes were the result of direct action of the parasites or of some toxic agent elaborated by them.

In the anterior or hemopoietic part of the kidney (Plate IV, fig. 7) and in the spleen, the only evidence of infection was indicated by the presence of massed siderin-like bodies among which fully formed spores were found. No effect on hemopoiesis was apparent in the kidney, mitotic figures being frequently seen. Some host response was indicated in the spleen by the presence of numerous macrophages with ingested granules and by a thickening of the fibrous capsule.

The damage to the liver was quite extensive. The lesions were characterized by a fibrosis and by a "waxy" or vacuolar-like degeneration of the hepatic cells (Plate IV, fig. 8). Large deposits of the yellow bodies were seen scattered throughout the liver lobules, especially in regions near

sinusoids. It should be pointed out that the fibrous tissue development was more extensive in those livers which were also infected with larval ascaroids.

The greatest accumulation of *H. visceralis* was found in the heart in extremely thin-walled cysts, probably developed in the subendocardial layer of areolar tissue (Plate V, figs. 9 and 10). These cysts were formed by a thin network of connective and vascular elements and contained numerous spores in various stages of maturation. No emboli were noted. There was, however, some degeneration of the myocardium and a definite thickening of the pericardium.

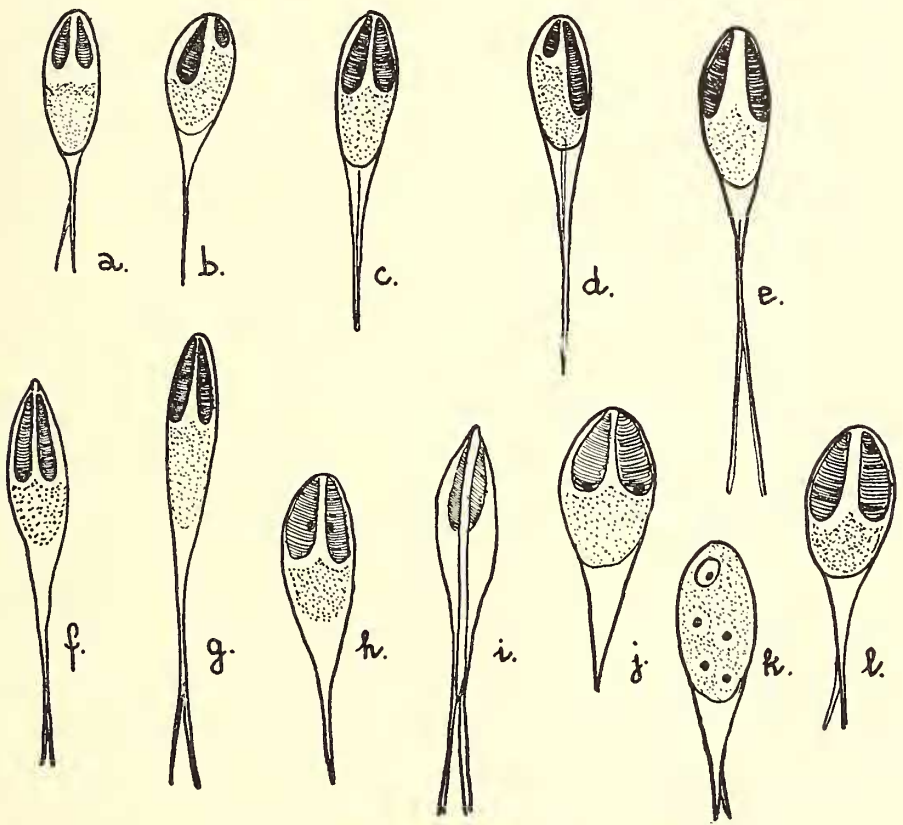
The spores of *H. visceralis* were also found in the mucous membranes of the mouth, but the associated pathology was not studied.

The spores of *H. electrica* occurred in cysts in the electric tissue (Plate VI, figs. 11 and 12). The only pathology noticed there was the destruction and replacement of comparatively large areas of the end-plate tissue, which comprises the tissue of the large electric organ. No inflammatory reaction was noted.

Discussion

Electrophorus electricus (Linnaeus) is a new host for the genus *Henneguya*. The species that it harbors possess features in which they differ from the other species of *Henneguya* described from Brazilian freshwater fish (Gurley, 1893; Cunha & Fonseca, 1918; Nemeczek, 1926; Pinto, 1928; Guimaraes, 1931; Guimaraes & Bergamin, 1933, 1934). This conclusion was reached after comparing both published figures and the measurements of the spore parts of the known species of *Henneguya* with those of the two new forms, named here *H. visceralis* and *H. electrica* spp. nov. (Table 1).

There is a dearth of information concerning the pathology of myxosporidiosis. In general, the tissue reactions are manifested by simple



Text-fig. 4. Unfixed mature and immature spores of *Henneguya* found in cysts on the skin (*d* to *f* and *h* to *l*) and in the mouth (*g*). Spores in figs. *a* and *c* are from the mucous scrapings from the mouth. Hanging drop preparation. Appr. 2000 ×. *a* and *c*—mature spores with short posterior extensions resembling *H. visceralis* sp. n. *b* and *d*—spores with asymmetrical polar capsules. *e*, *f* and *i*—forms resembling typical mature spores of *H. electrica* sp. n. *g*—a mature spore from a cyst in the mouth, probably a different species of *Henneguya*. *h*, *j*, *k* and *l*—forms resembling young spores of *H. electrica* sp. n.

cyst formation or fibrosis, and occasionally by a focal aceumulation of eosinophiles, lymphocytes and plasma cells. In the immediate regions of the infections, atrophy, hyalin degeneration and other necrobiotic changes are often present. In some instances, the host responses are characterized by a complete transformation in the character of the cells (Kudo, 1929, 1930 and 1946) and by a hyperplasia of the immediate and adjacent tissues (Nigrelli & Smith, 1938, 1940; Nigrelli, 1948). When the infection is diffused (i.e., when the parasites are not encysted), numerous siderin-like particles, either massed or scattered, are often associated with the developing parasites. It has been suggested (Doflein, 1898; also see Kudo, 1930, p. 321) that these yellow bodies are transformed secretory products of the parasite and that when present in large numbers they may cause death of the host. Since similar granules are known to occur in fishes with other types of infection, however, it is our belief that these yellow bodies represent degenerative products of the host tissue.

The occurrence of *H. visceralis* in the various tissues of the electric eel may indicate that the parasites are transported by the blood stream during some part of their life cycle. The kidney is the primary locus of infection and the parasites are probably disseminated by way of the renal and hepatic portal systems, since cysts were commonly found in the mesentery, liver, spleen and heart. The possibility, however, that the parasites may directly penetrate the larger blood vessels must also be taken into consideration.

Summary

Two new species of histozoic myxosporidians of the genus *Henneguya* are reported from a new host for protozoan parasites, *Electrophorus electricus* (Linnaeus); they are described under the names of *H. visceralis* sp. n. and *H. electrica* sp. n. The pathogenesis of this myxosporidiosis is described and discussed.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Accumulation of siderin-like deposits in the excretory portion of the kidney. Trophozoites and pansporoblasts of *Henneguya visceralis* are found among the interstitial elements and siderin-like deposits. Masson's stain. Approx. 250 ×.

Fig. 2. Nest of trophozoites of *H. visceralis* among kidney tubules and glomeruli. Note the sinusoids. Masson's stain. Approx. 500 ×.

Plate II

Fig. 3. Portion of posterior kidney showing tubular and glomerular degeneration and a number of uninucleate trophozoites of *H. visceralis*. Masson's stain. Approx. 800 ×.

Fig. 4. Cloudy swelling or parenchymatous degeneration of the kidney tubule and a group of siderin-like bodies including a mature spore of *H. visceralis*. Giemsa's stain. Approx. 1000 ×.

Plate III

Fig. 5. Surface cyst containing trophozoites of *H. visceralis* located on posterior kidney. Masson's stain. Approx. 120 ×.

Fig. 6. Parenchymatous degeneration in the excretory portion of the kidney. Masson's stain. Approx. 800 ×.

Plate IV

Fig. 7. Hemopoietic portion of the kidney showing intra- and extra-cellular siderin-like deposits among which mature spores of *H. visceralis* are found. Giemsa's stain. Approx. 1200 ×.

Fig. 8. Liver showing vacuolar-like degeneration and siderin-like deposits. Masson's stain. Approx. 600 ×.

Plate V

Fig. 9. "Nests" of *H. visceralis* spores in the endocardium of the ventricle. Note the thickened pericardium. Masson's stain. Approx. 10 ×.

Fig. 10. Phase contrast photomicrograph of living spores of *H. visceralis* from "nests" in the endocardium. Masson's stain. Approx. 800 ×.

Plate VI

Fig. 11. Cyst with mature spores of *H. electrica* in the electric tissue. Masson's stain. Approx. 250 ×.

Fig. 12. Phase contrast photomicrograph of unstained living spores of *H. electrica* from a cyst on the electric organ. Approx. 1000 ×.